the WT under mildly acidic conditions, but less efficiently upon further acidification. CD spectroscopy, intrinsic fluorescence and size-exclusion chromatography suggest that this mutant is more susceptible to acid destabilization and is prone to aggregation at neutral and mildly acidic pH. We suggest that the principal role of acidic residues in TH8-TH9 segment is not to modulate pH-dependent insertion directly, but to control the early stages of refolding in solution by protecting hydrophobic surfaces of the protein prior to initiation of membrane interactions. Supported by NIH GM-069783, Fulbright-CONICYT.

3633-Pos Board B361

Crucial Role of H322 in the Folding of Diphtheria Toxin T-Domain into the Open-Channel State

Mauricio Vargas-Uribe[1], **Mykola V. Rodnin**[1], Paul Kienker[2], Alan Finkelstein[2], Alexey S. Ladokhin[1].
[1]KUMC, Kansas City, KS, USA, [2]Albert Einstein College of Medicine, Bronx, NY, USA.

The translocation (T) domain plays a key role in the entry of diphtheria toxin into the cell. Upon endosomal acidification, the T-domain undergoes a series of conformational changes that lead to its membrane insertion and formation of a channel. Recently, we have reported that the triple replacement of the C-terminal histidines H322, H323 and H372 with glutamines prevents the formation of open channels in planar lipid bilayers. Here, we report that this effect is primarily due to the mutation of H322. We further examine the relationship between the loss of functionality and membrane folding in a series of mutants with C-terminal histidine substitutions using spectroscopic assays. The membrane insertion pathway for the mutants differs from that of the wild type as revealed by membrane-induced red-shift of tryptophan fluorescence at pH 6.0-6.5. T-domain mutants with replacements at H323 and H372, but not at H322, regain wild type-like spectroscopic signature upon further acidification. Circular dichroism measurements confirm that affected mutants misfold during insertion into vesicles. Conductance measurements reveal that substituting H322 dramatically reduces the numbers of properly folded channels in a planar bilayer, but the properties of the active channels appear to be unaltered. We propose that H322 plays an important role in the formation of open channels and is involved in guiding the proper insertion of the N-terminal region of the T-domain into the membrane. Supported by NIH GM069783 and in part by Fulbright Foundation (MVU).

Membrane Receptors and Signal Transduction IV

3634-Pos Board B362

Activation of Inhibitory G Protein Catalyzed by GPCR: Molecular Dynamics Simulations of the Activated Cannabinoid CB2 Receptor/Gαi1β1γ2 Protein Complex

Jagjeet Singh[1], Diane Lynch[1], Alan Grossfield[2], Nicholas Leioatts[2], Michael Pitman[1], Patricia Reggio[1].
[1]Dept of Chemistry & Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, USA, [2]Department of Biochemistry and Biophysics, University of Rochester Medical Center, Rochester, NY, USA.

The GPCR signaling cascade via the G protein pathway begins with an agonist binding to an inactive GPCR, causing conformational changes that activate the GPCR. Previously, we used molecular dynamics simulations to study the activation of the CB2 receptor (a Class A GPCR), by the endogenous ligand, 2-arachidonoylglycerol (2-AG) via the lipid bilayer (Hurst et al., 2010). In the next step of our study of the G-protein signaling cascade, we studied G-protein activation by an activated GPCR. In this step, GDP is released via the separation of the Gαi1 ras-like and helical domains of (Van Eps et al., 2011). To this end, we used our 2-AG activated CB2 model to produce an initial 2-AG/CB2/Gαi1β1γ2 complex based on the crystal structure of β2 adrenoreceptor in complex with Gαsβ1γ2 (Rasmussen et al., 2011). The complex was immersed in a fully hydrated 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer and NPT NAMD2 (Phillips et al., 2005) molecular dynamics (MD) simulations were initiated for four different trajectories of this system. Results from our longest MD simulation (3μs) suggest that the C-terminal α5 helix of Gαi prefers a different orientation in the CB2 activated receptor relative to the orientation seen in the empty state (GDP-GTP less) β2 adrenoreceptor/Gαsβ1γ2 complex. Initial hydrophobic interactions between P139 on CB2 intracellular loop 2 (IC-2 loop) and a hydrophobic pocket on Gαi consisting of residues V34 (N terminus); F336, T340, I343 and I344 (C terminus); L194 (β1-sheet); and F196 (β2-sheet) stabilize the receptor / Gαi1 protein interface during the first 0.5μs of the simulation. Later, between 1.4 - 1.6 μs, electrostatic interactions between R229 (CB2 IC-3 loop) and Q304/E308 (Gαi1 α4 helix) facilitate hydration of GDP. [Support: RO1 DA003934 and KO5 DA021358 (PHR)].

3635-Pos Board B363

Resolvin D1, a Trihydroxylated DHA Derivative, Displays Anti-Hyperreactive Effects on Human Pulmonary Arteries

Roddy Hiram[1], Edmond Rizcallah[2], Chantal Sirois[3], Caroline Morin[4], Eric Rousseau[5].
[1]Faculté de Médecine et Sciences de la Santé, Université de Sherbrooke, Sherbrooke, QC, Canada, [2]Pathology, CHUS, Sherbrooke, QC, Canada, [3]Chirurgie Thoracique, CHUS, Sherbrooke, QC, Canada, [4]UQAR, SFPharma, RIMOUSKI, QC, Canada, [5]Physiology and Biophysics, Université de Sherbrooke, Sherbrooke, QC, Canada.

Pulmonary Hypertension (PH) is a rare and progressive disease characterised by an inflammatory status and vessel wall remodeling, resulting in an increased pulmonary arteries resistance. In the last 20 years, pharmacological treatments have been proposed. However PH remains associated with an important morbidity. Recent studies demonstrate that omega-3 fatty acid derivatives, such as docosahexaenoic acid monoacylglyceride (MAG-DHA) displays anti-inflammatory and modulatory electro-physiological effects (Morin et al., 2008). The goal of this project is to evaluate the sensitivity of human pulmonary arteries (HPA) to omega-3 derivatives and to assess the effects of Resolvin D1 (RvD1), on their pharmacological reactivity. Specific Objectives: 1) To asses the effects of RvD1 and docosapentanenoic acid monoacylglyceride (MAG-DPA) on the mechanical tension and Ca^{2+} sensitivity, developed by HPA treated or not with 5 nM endothelin-1 (ET-1). 2) To test the effects of KCl, 5-HT, U-46619 and PDBu Under various experimental conditions. 3) To evaluate the mode of action of RvD1 and MAG-DPA on the expression and phosphorylation level of various proteins. Our results demonstrate that 5 nM ET-1 pretreatment during 24 h increased the reactivity and Ca^{2+} sensitivity of HPA. 300 nM RvD1 pretreatment decreased the hyper-reactivity induced by ET-1 and also decreased the pharmacological responses. 1μM MAG-DPA pretreatment decreased the mechanical tension induced by ET-1 and the agonists. RvD1 and MAD-DPA also decreased the expression of TMEM 16 A and the phosphorylation level of CPI-17 protien. Thus we demonstrate for the first time, that RvD1, a well known antiinflammatory compound displays modulatory effects on HPA contractile properties.

3636-Pos Board B364

Quantitative Analysis of Receptors and Second Messengers Interactions in Pancreatic Beta Cell

Leonid E. Fridlyand, Louis H. Philipson.
University of Chicago, Chicago, IL, USA.

The response of insulin secretion in pancreatic beta cell to nutrient stimuli and hormonal modulators is coordinated by the different receptors, messengers and signaling networks. We present an updated computational model of second messenger interactions in pancreatic beta cell that incorporate modern data on glucose metabolism, plasma membrane potential, G-protein-coupled-receptors (GPCRs), cytoplasmic and endoplasmic reticulum calcium dynamics, cAMP and phospholipase C pathways. Model includes glucagon like peptide 1 receptor, gastric inhibitory polypeptide receptor and adrenoreceptor for cAMP pathway regulation, and the muscarinic acetylcholine receptor and the fatty acid receptor (GPR40) for phospholipase C regulation. The values of most of the model parameters were inferred from available experimental data. Our analysis of the dynamic data provides evidence for a pivotal role for Ca^{2+}-dependent adenylyl cyclase activation in the effect of glucagon-like peptide 1 on pancreatic β-cells. The regulatory properties of various adenylyl cyclase isoforms determine fluctuations in cytoplasmic cAMP concentration and reveal a synergistic action of glucose and glucagon-like peptide 1 on insulin secretion. On other hand, the regulatory properties of phospholipase C isoforms determine interaction of glucose, acetylcholine and fatty acids (that act through the receptor GPR40). We test the hypothesis that activation of specific key beta-cell GPCRs can be in some cases stimulate but in other combinations inhibit glucose-stimulated insulin secretion. The regulation of messenger's pathway interactions may be important pharmacological targets for improving insulin secretion in type 2 diabetes.

3637-Pos Board B365

Role of Akaps in BCAM/Lu Receptor Activation on Normal and Sickle Erythrocytes

Jamie Maciaszek[1], Biree Andemariam[2], **George Lykotrafitis**[1].
[1]Department of Mechanical Engineering, University of Connecticut, Storrs, CT, USA, [2]Sickle Cell Center, University of Connecticut Health Center, Farmington, CT, USA.

Background: Human normal and sickle red blood cells (RBCs) adhere with high affinity to laminin-5 via the basal cell adhesion molecule/Lutheran (BCAM/Lu) receptor which is implicated in vasoocclusive episodes (VOEs) in sickle cell disease (SCD). BCAM/Lu is activated through the cyclic adenosine monophosphate (cAMP) signaling pathway.